U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-252 814-T

INTESTINAL CU ABSORPTION IN VITRO AFTER FE OR ZN DEPLETION

ENVIRONMENTAL PROTECTION AGENCY

1973

141039

PB 252 814 -T

EPA - TR 374-74

F. J. Schwarz, M. Kirchgessner

INTESTINAL CU ABSORPTION IN VITRO AFTER FE OR ZN DEPLETION

[Intestinale Cu-Absorption in vitro nach Fe- oder Zn-Depletion]

Z. Tierphysiol., Tierernährg. u. Futtermittelkde,
Vol. 31, 1973, pp. 91-98

Translated for the
ENVIRONMENTAL PROTECTION AGENCY
by
Translation Consultants, Ltd., Arlington, Virginia

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

BIBLIOGRAPHIC DATA SHEET	1. Report No. EPA TR74-374	2.	3. Recipient's Accession No. PB-252 814-T
4. Title and Subtitle Intestinal CU absorption in vitro after FE or ZN Depletion			5. Report Date 1973 6.
7. Author(s) F.J. Schwarz, M. Kirchgessner			8. Performing Organization Rept. No.
9. Performing Organization Name and Address Translated from theGerman by EPA			10. Project/Task/Work Unit No. 11. Contract/Grant No.
12. Sponsoring Organization Name and Address EPA ISD Translation Services Division Library RTP, NC 27711			13. Type of Report & Period Covered 14.

15. Supplementary Notes

Z. Tierphysiol., Tierernahrg. u. Futtermittelkde 31:91-98 1973

16. Abstracts

17. Key Words and Document Analysis. 17a. Descriptors

Trace Elements
Radiobiology in vitro analysis
EPA ISD

17b. Identifiers/Open-Ended Terms

Cu Absorption
Iron
Zinc

17c. COSATI Field/Group 06-C

PRICES SUBJECT TO CHANGE

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
Exclusive permissiongranted to NTIS for the Storage and distribution of this material.	UNCLASSIFIED	17
	20. Security Class (This Page) UNCLASSIFIED	22. Price $3.50-2.25

F. J. Schwarz, M. Kirchgessner*

INTESTINAL CU ABSORPTION IN VITRO AFTER FE OR ZN DEPLETION

The organism seeks to adapt to a deficient supply of trace elements by increased absorption. It was possible to primarily demonstrate this for iron (Forth and Rummel, 1971) as well as for copper and zinc (Kirchgessner, Schwarz and Grassmann, 1972). Of course the matter is not yet clear whether the improved absorption with depletion is specific for the actual trace element or whether other trace elements, too, are increasingly absorbed. For this reason, Cu absorption in vitro after Fe or Zn depletion of rats is tested in the present investigations with everted intestinal sacs.

Experimental Method

1. Test Animals

Weaned male Sprague-Dawley rats were used as test animals. The animals were kept in groups in plastic cages made of macralon. For the Fe depletion experiment, 42 rats were divided into 6 groups (7 animals per group) according to their same average weight (50 g). Three groups received

---

* Eleventh Report on the Dynamics of Copper Absorption.
Manuscript received 4 September 1972.

a casein diet ad libitum containing 8 mg iron/kg. The remaining animals had 20 mg iron/kg added to their food (Grassmann and Kirchgessner, 1973). For the Zn depletion experiment, 8 rats ranging in weight from 40 to 45 g received ad libitum a low zinc diet (approximately 2 mg zinc/kg) as well as Zn-free drinking water (Pallauf and Kirchgessner, 1971). Two control groups, each consisting of 6 animals, were fed ad libitum with the same diet to which, however, 20 or 60 mg zinc/kg had been added.

2. Test Procedure

The rats were starved from 14 to 16 hours and decapitated under ether narcosis at the end of the depletion period. The preparation of the intestine and further test procedure corresponded to the method of everted intestinal sacs (Schwarz and Kirchgessner, 1972; Wilson and Wiseman, 1954). A 50 cm long section of the small intestine distal from the Flexura duodenojejunalis was removed from each animal. It was everted and preincubated for five minutes in Tyrode's solution and tied off to an intestinal sac. Tyrode's solution (pH 7.0) was used as serosa solution. The filling of the intestinal sac varied according to the sac's volume for the individual test groups. The intestinal sacs were incubated in 50 ml Tyrode's solution (pH 5.6) to which 10^{-4} mol copper/l had been added as $CuSO_4$. Oxygen was passed into the mucosa solution and the incubation

vessels were uniformly agitated (30 rpm) in the water bath (37° C) during the entire test (60 minutes).

The intestinal sacs had to be washed after incubation in a physiological NaCl solution containing 10^{-2} mol EDTA/l (Forth and Rummel, 1966) in order to separate the coarsely absorptive bound copper from the intestinal wall. Cu content of the serosa solution and, thus, of the Cu transferred through the intestinal wall, was determined using atomic absorption spectrophotometry (AAS) (Allan, 1961). After determination of the intestinal wall dry substance (drying at 105° C to point of constant weight), the latter was reduced to ash in a muffle furnace at 450° C and Cu content was measured in 0.1 N HCl with the AAS.

Findings

1. Cu Absorption after Fe Depletion

Test animals from an Fe-anemic and a control group, respectively, were decapitated after a 10, 15 or 22 day depletion period. At the same time, Hb value of the blood was determined (Grassmann and Kirchgessner, 1973). Measurements are listed in Table 1. The average rate of the depletion groups did not differ in comparison to the control groups. The mean live weight after 10 days amounted to 74 g, after 15 and 22 days, 93 or 115 g. Corresponding to the differing weights, after 10 days 3 ml, and finally

4 ml, of serosa solution was poured into the intestinal sac. Table 1 shows the concentrations for the Cu transfer into the serosa liquid and Cu absorption by the intestinal wall. The values provide the standard deviation of the individual values in the following table.

Table 1

Cu transfer into the serosa solution and Cu absorption by the intestinal wall after Fe depletion of the test animals

Test duration	10 days		15 days		22 days	
Preliminary treatment	Depleted	20 ppm iron	Depleted	20 ppm iron	Depleted	20 ppm iron
Hb value (g/100 ml)	7.4 ±0.6	10.1 ±0.2	5.9 ±0.4	10.0 ±0.5	4.2 ±0.7	11.8 ±0.6
Cu transfer into serosa solution, µg Cu total	0.69 ±0.28	0.49 ±0.14	0.50 ±0.33	0.72 ±0.28	0.51 ±0.15	0.60 ±0.32
Cu absorption by intestinal wall, µg Cu total	93.1 ±5.6	102 ±13.0	90.1 ±10.2	93.6 ±6.7	84.4 ±3.7	88.8 ±6.0
µg Cu/g DM	281 ±11	291 ±47	249 ±40	243 ±24	232 ± 15	213 ±30
Intestinal wall g DS	0.33 ±0.01	0.35 ±0.02	0.36 ±0.03	0.39 ±0.02	0.37 ±0.02	0.43 ±0.05

The results were evaluated using the bifactorial variants analysis according to the Fe supply and depletion duration. Insofar as the anemic animals were concerned, no difference was seen as compared to the control animals in the height of

Cu transfer into the serosa fluid even after a lengthy period of depletion. With an increasing length of preliminary treatment, however, Cu absorption by the intestinal wall decreases both for the depleted, as well as for the Fe-supplied animals ($P < 0.05$). Relative to g of dry substance (DS) of the intestinal wall, the effect increases ($P < 0.001$), because the weight of the intestinal section increases with increasing age. The intestinal sections supplied with Fe absorb more copper ($P < 0.05$) in contrast to the anemic rat intestines. However, this effect could no longer be determined in the case of Cu contents, relative to g DS of the intestinal wall, because the isolated intestinal sections of anemic animals displayed a somewhat lesser weight.

2. Cu Absorption after Zn Depletion

After a 30-day preliminary treatment of the test animals with the low zinc level diet the average weight of the depletion group amounted to 51 g, the control groups with 20 mg zinc/kg diet to 202 g, and with 60 mg zinc/kg to 218 g (Roth, 1972). 2.5 ml serosa solution was poured into the depleted intestinal sacs and 5 ml serosa solution into the sacs of Zn-supplied animals because of the different intestinal volume. Mean values for the Cu transfer into the serosa fluid and for Cu absorption by the intestinal wall are listed in Table 2.

Table 2

Mean Cu transfer into serosa solution and Cu absorption by the intestinal wall after Zn depletion of the test animals

Preliminary treatment	Zn depletion	Control groups 20 mg Zn	60 mg Zn
Cu transfer in serosa solution, µg Cu total	0.66 ±0.13	0.45 ±0.20	0.47 ±0.10
µg Cu/ml serosa solution	0.27 ±0.05	0.09 ±0.04	0.09 ±0.02
µg Cu/g DS intestinal wall	3.34 ±0.91	0.80 ±0.32	0.87 ±0.19
Cu absorption by the intestinal wall, µg Cu total	67.2 ± 7.5	91.8 ±6.2	95.3 ±4.9
µg Cu/g DS	335 ±49	166 ±11	176 ±24
Intestinal wall, g DS	0.20 ± 0.03	0.56 ±0.04	0.55 ±0.04

Although the absorption surface of the jejunum of the larger animals is greater for the same intestinal length (50 cm), Cu quantity on the serosa side is already significantly increased ($P < 0.05$) in the depleted rats. When Cu concentration in the serosa fluid is taken into consideration or absorption is related to g DS of the intestinal wall, the values are approximately 3 to 4 times higher than the values for Zn-depleted animals ($P < 0.001$).

Discussion

Trace elements are better absorbed with minimal application (Forth and Rummel, 1971; Van Campen, 1971; Becker and Hoekstra, 1971). One series of experiments showed that anemic animals absorbed more iron than normal animals (Bothwell et al., 1958; Brown and Rother, 1963; Wheby et al., 1964). It was also possible to confirm these findings in vitro by means of Fe-depleted sections of the small intestine (Manis and Schachter, 1962; Ruliffson and Hopping, 1963; Forth and Rummel, 1965; Helbock and Saltman, 1967). Kirchgessner et al. (1972) achieved with everted intestinal sacs of Cu-depleted rats a 50 to 80% higher Cu transfer and increased Cu absorption than for rats with controlled Cu supply. It was also possible to observe higher absorption in Cu-depleted sheep (Neethling et al., 1968) and improved retention in rats (Owen, 1971). Absorption in vitro of the trace element zinc was approximately 3 times higher after Zn depletion (Kirchgessner et al., 1972).

1. Cu Absorption and Fe Depletion

Improved absorption following previous depletion can be used for the purpose of investigating the specificity of possible transport systems for trace elements in the case of transfer through the absorption cell layer. With in vitro as well as in situ experiments on rats, it was possible

to find in Fe deficiency a higher absorption not only of iron but also of cobalt, manganese, nickel and zinc (Forth and Rummel, 1971). On the other hand, the absorption rate of copper remained unchanged. The work of Pollack et al. (1965) also stands in complete agreement. It was not possible for them to increase Cu absorption in situ in the case of Fe-depleted rats although iron, cobalt, manganese and zinc was increasingly absorbed. It was possible to confirm these observations for copper on Fe-depleted rat intestines in the present work. The in vitro Cu absorption of three test groups which were depleted with a low Fe diet, showed no differences as compared to the control groups. Actually, Cu absorption by the intestinal wall was somewhat reduced in the depleted animals. A lesser Cu bond in Fe-depleted intestinal sections in vitro was indicated also by Forth and Rummel (1971). However, this could be based on the somewhat lesser intestinal weights of the anemic rats found in the present experiments.

Copper becomes concentrated quite strongly in the liver with Fe deficiency (Sourkes et al., 1968; Grassmann and Kirchgessner, 1973). This increased liver-Cu content, therefore, does not appear to be related to improved Cu absorption. Rather, according to Forth and Rummel (1972; Forth et al., 1968), the different behavior in absorption and in transfer of copper and iron is explained by the fact that reticulocytes

absorb more iron, cobalt, manganese and zinc than erythrocytes, the same not being true with copper. Further, Schade et al. (1970) found it possible to decrease Fe absorption with an excess of cobalt, whereas absorption of copper remained unchanged. In the discussion of these differences between copper and iron, we should include the heavy metal bonding proteins of the mucosa which recently have been isolated a number of times (Starcher, 1969; Evans and Cornatzer, 1970; Hubers et al., 1971; Suso and Edwards, 1971; Worwood et al., 1971).

2. Cu Absorption and Zn Depletion

In the second experiment series of the present work, Cu absorption of Zn-depleted rats was measured in comparison to normally supplied rats. Cu transfer was significantly increased in the intestinal sections, which were taken from the animals with Zn deficiency. The increase of Cu transfer (relative to g DS intestinal wall) into the serosa fluid was approximately four times higher than in the control groups. Cu absorption by the intestinal wall doubled in the case of the Zn-depleted rats. Indeed, the experiment to determine Cu absorption after Fe depletion disclosed that the age, or live weight, of the animals have a minimal effect on Cu absorption.

A series of interactions in the organism were observed between copper and zinc (Underwood, 1971). Indeed, the interactions appearing must always be considered in connection with the basic experimental conditions; i.e., with the state of supply of the animal and the diet administered. With Cu-deficient animals, zinc quantities adequate to requirements could already produce toxic effects which can be considered an intensification of the symptoms of Cu deficiency (Hill and Matrone, 1962; Savage et al., 1963). On the other hand, Cu toxicity can be reduced through increased supply of Zn (Ritchie et al., 1963; Suttle and Mills, 1966). In the case of normal Cu supply, extremely high additions of Zn decrease the Cu storage in the liver (Grant-Frost and Underwood, 1958; Magee and Matrone, 1960; Johnson et al., 1962).

Since in the case of Zn-depleted rats, both zinc (Kirchgessner et al., 1972) as well as copper can be increasingly absorbed and transferred, it can be concluded that there are similarities in Cu and Zn absorption and bonding in the intestinal wall. Starcher (1969) was able to show that zinc and copper compete for the same protein which appears to be responsible for the copper bond in the duodenum of young chicks. Even the protein isolated by Evans and Cornatzer (1970) in the duodenum of the rat bonds zinc and copper in the same way. Van Campen and Scaife (1967) and Van Campen (1969) already explained the interactions

between copper and zinc on the basis of mutual effects in the intestinal tract or during absorption. For example, large Zn supplies simultaneously administered into the intestinal volume inhibited Cu absorption while high Cu delivery decreased Zn absorption. On the other hand, a previous or simultaneous intraperitoneal delivery of the antagonist did not affect either Cu or Zn absorption.

Summary

Cu absorption after Fe or Zn depletion was measured in vitro with everted sacs of rats. The experimental animals were depleted with a casein diet low in Fe or in Zn.

1. The Cu transfer to the serosa solution of anemic intestines remained unchanged compared with that of Fe-supplied intestinal segments; only after Cu depletion the Cu transfer was 50-80% higher.

2. In Zn deficiency the Cu transfer to the serosa solution as well as the Cu uptake by the intestinal wall (based on g DS of intestinal wall) was clearly elevated ($P < 0.001$). Since after Zn depletion the Zn absorption was elevated about threefold possible interactions between zinc and copper in the uptake and transfer of these trace elements are discussed.

Bibliography

Allan, J.E., Spectrochim. Acta 17, 1961, p. 459.

Becker, W.M., W.G. Hoekstra, Intestinal Absorption of Metal Ions, Trace Elements and Radionuclides, (Skoryna, S.C., and D. Waldron-Edward), Pergamon Press Oxford, New York, 1971, p. 229.

Bothwell, T.H., G. Pirzio-Biroli, C.A. Finch, J. Lab. Clin. Med. 51, 1958, p. 24.

Brown, E.B., M.L. Rother, J. Lab. Clin. Med. 62, 1963, p. 804.

Evans, G.W., W.E. Cornatzer, Federation Proc. 29, 1970, p. 695.

Forth, W., W. Rummel, Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. 252, 1965, p. 205.

_____, Med. Pharmacol. exp. 14, 1966, p. 289.

_____, Intestinal Absorption of Metal Ions, Trace Elements and Radionuclides, (Skoryna, S.C., and D. Waldron-Edward), Pergamon Press Oxford, New York, 1971, p. 173.

_____, Pharmacology of Gastrointestinal Absorption, (Rummel, W. and W. Forth), Pergamon Press Oxford, New York (in press), 1972.

Forth, W., W. Rummel, D. Crusemann, J. Simon, Stoffwechsel und Membranpermeabilitat von Erythrocyten und Thrombocyten, (Deutsch, F., F. Gerlach, and K. Moser), Georg Thieme Verlag, Stuttgart, 1968, p. 444.

Grant-Frost, D.R., F.J., Underwood, Austral, J. Exptl. Biol. Med. Sci. 36, 1958, p. 339.

Grassmann, E., M. Kirchgessner, Z. Tierphysiol., Tierernahrg. u. Futtermittelkde. (in press), 1973.

Helbock, H.J., P. Saltman, Biochim. Biophys. Acta 135, 1967, p. 979.

Hill, C.H., G. Matrone, Proc. 12th World's Poultry Congress, Sydney, Sect. Papers 12, 1962, p. 219.

Hubers, H., F. Hubers, W. Forth, W. Rummel, Life Sci. 10, 1971, p. 1141.

Johnson, D., Mehring, A.L., Savino, F.X., Titus, H.W., Poultry Sci. 41, 1962, p. 311.

Kirchgessner, M., F. J. Schwarz, E. Grassmann, Bioorganic chem. 2, 1972, p. 169.

Magee, A.C., G. Matrone, J. Nutr. 72, 1960, p. 233.

Manis, J.G., D. Schachter, Amer. J. Physiol. 203, 1962, p. 81.

Neethling, I.P., J.M. Brown, P.J. Wet, J.S. African Vet. Med. Assoc. 39, 1968, p. 13.

Owen, C.A., Jr., Amer. J. Physiol. 221, 1971, p. 1722.

Pallauf, J., M. Kirchgessner, Z. Tierphysiol., Tierernahrg. u. Futtermittelkde. 28, 1971, p. 128.

Pollack, S., J.N. George, R.C. Reba, R.M. Kaufman, W.H. Crosby, J. Clin. Invest. 44, 1965, p. 1470.

Ritchie, H.D., Luecke, R.W., Baltzer, B.V., Miller, F.R., D.E. Ullrey, J.A. Hoefer, J. Nutr. 79, 1963, p. 117.

Roth, P., (Unpublished), 1972.

Ruliffson, W.S., J.M. Hopping, Amer. J. Physiol. 204, 1963, p. 171.

Savage, J.F., D.W. Bird, B.L. O'Dell, Poultry Sci. 42, 1963, p. 1304.

Schade, S.G., B.F. Felsher, G.M. Bernier, M.E. Conrad, J. Lab. Clin. Med. 75, 1970, p. 435.

Schwarz, F.J., Kirchgessner, M., Intern. Z. Vit. Ern. Forschung 42, 1972, p. 592.

Sourkes, T.L., K. Lloyd, H. Birnbaum, Canad. J. Biochem. 46, 1968, p. 267.

Starcher, B.C., J. Nutr. 97, 1969, p. 321.

Suso, F.A., H.M. Edwards, Proc. Soc. Exp. Biol. Med. 137, 1971, p. 306.

Suttle, N.F., C.F. Mills, Brit. J. Nutr. 20, 1966, p. 135.

Underwood, E.J., Trace Elements in Human and Animal Nutrition. 3., Ed. Academic Press, New York and London, 1966.

Van Campen, D.R., P.U. Scaiff, J. Nutr. 91, 1967, p. 473.

Van Campen, D.R., J. Nutr. 97, 1969, p. 104.

_____, Intestinal Absorption of Metal Ions, Trace Elements, and Radionuclides, (Skoryna, S.C. and D. Waldron-Edward), Pergamon Press Oxford, New York, 1971, p. 211.

Wilson, T.H., G. Wiseman, J. Physiol. 123, 1954, p. 116.

Wheby, M.S., L.G. Jones, W.H. Crosby, J. Clin. Invest. 43, 1964, p. 1433.

Worwood, M., A. Edwards, A. Jacobs, Nature 229, 1971, p. 409.